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UNUS #: Y2-R566-EDG

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Pillai et al.
Serial No.: 10/003,850
Filed: November 2, 2001
For: SKIN CARE PRODUCT CONTAINING RETINOIDS, RETINOID BOOSTER AND PHYTOESTROGENS IN A DUAL COMPARTMENT PACKAGE

Group: 1617
Examiner: R.S. Travers
Edgewater, New Jersey 07020
April 26, 2004

DECLARATION UNDER 37 CFR 1.132

Assistant Commissioner for Patents
Alexandria, VA 22313-1450

Sir:

I, Susanne Teklits Iobst, residing at 89 Stelling Avenue, Maywood, NJ 07607 do hereby declare that:

1. I am a citizen of the United States.

2. My educational and technical background in the field of Biochemistry is as follows:

(a) I received a Bachelor of Science Degree in Biochemistry from Lehigh University in 1986.

(b) I received a Master of Science Degree in Chemistry from Stevens Institute of Technology in 1989.

(c) I received a Doctorate of Philosophy from the Department of Biochemistry and Molecular Biophysics at Columbia University in 1995.

(d) I joined my present employer Unilever in 1986 and I currently have the title Research Scientist, located in Edgewater, NJ.

3. I have read Pillai et al., US Patent Application no. 10/003,850 filed November 2, 2001.

4. The following experiments were conducted through an external collaboration in support of the above-cited Pillai et al. patent application.

5. **Transglutaminase Assay**

Based on the data presented in this validation document, the standard protocol that will be adapted for testing the active compounds for synergistic inhibition of transglutaminase production in retinol treated keratinocytes is as follows:

Final Assay Concentrations

Keratinocytes	3000 cells/well
Retinol	10^{-7}M
Active Compounds	IC_{20} based on individual dose response curves to facilitate identification of synergistic inhibition.

The keratinocyte assay plates were incubated for 72 hours with the test compounds in a total volume of 200 µl at 37°C, 5% CO_2, in the incubator. At the end of the incubation period, the media is completely removed from each well. The wells are rinsed twice with 1xPBS. The plates are then frozen at –70°C. After thawing the plates, they are then incubated sequentially with blocking buffer (1 hour), primary antibody (TGm-specific monoclonal antibody B.C1 diluted 1:2000 in washing buffer) (2 hours) and secondary antibody (peroxidase labeled antimouse IgF(ab)2 fragment diluted 1:4000 in washing buffer) (2 hours). Each step is followed by rinsing the wells 3X with washing buffer. The plates are then incubated with 100 µl/well substrate solution at room temperature for exactly 5 minutes. The reaction is stopped with 50 µl/well 4N H_2SO_4. The absorbance is read at 490 nm in a Bio-tek absorbance plate reader.

Results: Table 1

	% TG production	% inhibition
Phosphatidyl choline	97.7%	2.3%
Glycyrrhetinic Acid	92.1%	7.9%
Phosphatidyl choline + Glycyrrhetinic acid	73.1%	26.9%

Note: The greater the inhibition the more effective the compound.

6. I conclude the following from these experiments:

Based on the analysis above the combination of a B2 and B3 booster gives a synergistic effect in the presence of retinol for the inhibition of transglutaminase expression. As the size of the effect is greater than additive it is a strongly synergistic result.

7. I declare that all statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under ə 1001 of Title 18 of the United States Code and may jeopardize the validity of the application or any patent issuing thereon.

Dated: April 23, 2004

By: Susanne Teklits Lobst
Susanne Teklits lobst

Title: Research Scientist